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Experimental evidence for a causal effect of pair-bond duration on reproductive performance in oystercatchers (*Haematopus ostralegus*)

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Many studies have suggested that reproductive performance improves during the pair-bond, which might explain why individuals remate with the same partner in many species. However, discussion exists about whether the association between reproductive performance and pair-bond duration that is reported in these studies reflects a causal relationship. Usually it is unclear whether a positive association is caused by pairs improving during their pair-bond or by high-quality pairs staying together for longer. Furthermore, reproductive performance often also depends on the age or breeding experience of parents, which all covary with pair-bond duration. A much needed experimental approach is lacking so far. We investigated the effect of pair-bond duration on reproductive performance in a long-lived monogamous bird species based on natural as well as experimental variation. The duration of oystercatcher (*Haematopus ostralegus*) pair-bonds, which were followed for 21 years, strongly affected reproductive output, even after controlling for effects of age and breeding experience. Pairs improved during their pair-bond, and there were no indications of selective disappearance of low-quality pairs; however, pairs that stayed together for very long performed badly. Experimental removal of one partner showed that the reproductive cost of divorce depended on the pair-bond duration with the old partner. In addition, after remating, the newly formed pairs strongly improved again, independent of the age and breeding experience of the remated pair members. As such, this study provides the first experimental evidence of a causal effect of pair-bond duration on reproductive performance. *Key words:* age-dependent reproduction, constraint, divorce, intrapair cooperation, long-term monogamy, restraint. *[Behav Ecol 17:982–991 (2006)]*

Reproductive performance of iteroparous animals usually changes systematically during a lifetime (Clutton-Brock 1988; Newton 1989). At least 30 studies, mainly on socially monogamous bird species, have suggested that changes in reproductive performance during a lifetime are (partly) caused by pairs improving during their pair-bond (reviewed by Fowler 1995; Black 1996a). Such a "mate familiarity effect" (Black 1996b) is important for our understanding of the evolution of long-term monogamy, as it might explain why in many species individuals remate with the same partner every year. Furthermore, it suggests pair members are more able (or willing) to cooperate during their pair-bond. Understanding the mechanism behind improved cooperation might shed light on the evolution of cooperation in repeated social interactions (Axelrod 1984).

Several authors have noted that a correlation between pair-bond duration and reproductive performance at the population level does not necessarily imply a causal relationship (e.g., Forslund and Pärt 1995; Ens et al. 1996). First of all, reproduction is also often correlated with the age or breeding experience of males and females. Pair-bond duration usually strongly covaries with age and breeding experience, as pair members that have been together for very long are by definition also old and experienced breeders. Therefore, a statistical effect of pair-bond duration on reproductive performance might actually reflect a correlated causal change in one of these other age-related parameters (the age or breeding experience of the male and female). Several studies have used multiple regression techniques to distinguish between effects of age-related parameters and pair-bond duration (Fowler 1995; Black 1996a, and references therein); and 2 studies simultaneously investigated all age-related parameters of both sexes (Bradley et al. 1990; Pyle et al. 2001). These studies suggest that even after statistically correcting for age effects, pair-bond duration remained important. However, the power of a statistical approach to accurately disentangle strongly intercorrelated effects is often limited (Graham 2003). Reliable interpretation of the separate effect of pair-bond duration in a multiple regression approach can be strongly confounded by even low levels of multicollinearity with age and breeding experience (Ens et al. 1996); very large sample sizes are required to overcome this problem.

A second type of alternative explanations suggests that a correlation at the population level between pair-bond duration and reproductive performance might reflect progressive changes in the phenotypic composition of a population (e.g., Black 2001). For example, low-quality pairs might divorce more often (or die younger), resulting in pairs with long pair-bonds being of high phenotypic quality. Such selective divorce or widowing can result in a correlation at the

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population level, without any improvement in reproductive performance within pairs.

Because few studies investigated all alternative explanations (but see Bradley et al. 1990; Pyle et al. 2001), some authors have questioned the overall evidence for an improvement of reproductive performance during the pair-bond (discussed by Cézilly and Nager 1995; Pärt and Forslund 1995; Ens et al. 1996). They argued experimental control is crucial because a statistical approach cannot correct for possible important unmeasured parameters that are correlated with pair-bond duration (e.g., individual quality, breeding site fidelity).

In this study, we investigated whether pairs of the long-lived monogamous oystercatcher (*Haematopus ostralegus*) improved reproductive performance during their pair-bond using both an observational as well as an experimental approach. We first investigated the effect of pair-bond duration on reproductive performance in a large group of pairs, which were followed up to 21 years. We statistically controlled for confounding effects of other age-related parameters of the male and female parents. In addition, we investigated whether a correlation between pair-bond duration and reproductive performance was caused by selective divorce (or widowing) of low-quality pairs. Subsequently, we experimentally tested whether pair-bond duration affects reproductive performance by removing birds, forcing the remaining partner to remate. We investigated whether the reproductive cost of divorce depended on the pair-bond duration with the removed partner. In addition, we investigated whether the newly formed pairs improved their reproductive performance in the 4 years after remating.

MATERIALS AND METHODS

From 1983 until 2003, we followed a population of approximately 100 breeding pairs on Schiermonnikoog (32°29′N 6°14′W), an island in the Dutch Wadden Sea. All breeders and their fledglings were color banded, as well as a large part of the nonbreeders. Every year we followed the breeding performance of all pairs (protocol see van de Pol, Bakker et al. 2006). Oystercatchers breed on the salt marsh and feed on the adjacent intertidal mud flats. Following earlier studies, we designated territories with adjacent feeding and nesting areas as high-quality territories and territories with spatially separated feeding and nesting areas as low-quality territories (Ens et al. 1992). Pairs with high-quality territories (residents) consistently produce 2–3 times more fledglings each year than pairs in low-quality territories (leapfrogs). Both male and female oystercatchers contribute about equally to the parental care of their semi-precocial offspring. Birds were sexed using DNA analysis (Heg, Dingemanse, et al. 2000), biometric measurements (Zwarts et al. 1996), and observations of copulations (Heg et al. 1993).

Pair-bond duration, age, and breeding experience

Oystercatchers are long-lived, socially and genetically monogamous, and have high breeding site fidelity (Heg et al. 1993, 2003; Heg, Ens, et al. 2000). Age at first reproduction is extremely variable (range 3–11 years); established breeders display some intermittent breeding (Bruinzeel 2004). Annual resighting probability of breeders in this population is virtually one.

Analyses were restricted to birds with exact known pair-bond duration, that is, pairs that were ringed at the start of the study were excluded. Only a part of the breeding population was of known age (16%) because only individuals ringed as fledgling or juveniles could be exactly aged on morphological characteristics (Prater et al. 1987). The age and breeding experience of known-aged individuals were highly intercorrelated within both sexes (♂: $r = 0.83$, ♀: $r = 0.82$). However, due to frequent divorce (8% annually) and widowing (7% mortality annually), correlations between pair-bond duration and age (or breeding experience) of males or females were less strong ($r = 0.4$–0.5). Because collinearity between age and breeding experience were extremely high, we decided to include only effects of male and female breeding experience and pair-bond duration in the analyses. We preferred to include effects of breeding experience over age effects because breeding experience could also be estimated for individuals of unknown age. Many breeders were already banded when they were a nonbreeder, which allowed exact determination of the amount of breeding experience. In addition, we assumed that unbanded birds entering the breeding population had no previous breeding experience because many breeders in surrounding areas were also color banded and breeding dispersal is very local (van de Pol, Bruinzeel et al. 2006). To verify that estimated breeding experience was an unbiased estimator of real breeding experience, we calibrated estimation rules on individuals that were ringed as juveniles. Estimated breeding experience predicted exact breeding experience very well ($r = 0.88$). Although age effects were omitted from analyses, interpretations of effects of breeding experience also encompass age effects.

Reproductive parameters

We omitted all experimentally manipulated pairs and pairs that could not be assigned to a nest with complete certainty. To quantify the effect of pair-bond duration on annual reproductive output, we used the annual number of fledglings produced by each pair. To investigate effects of pair-bond duration on different stages of the breeding cycle, reproductive performance was further partitioned into laying date, clutch size, egg survival, and chick survival (Table 1). Note that fledgling production is the result of clutch size × egg survival × chick survival; laying early is strongly associated with high fledgling production, as in most bird species. Clutch size and fledgling production were known for 1224 pair years; records for other reproductive parameters were less complete (Table 1). We analyzed laying date, clutch size, egg survival, and chick survival of first clutches only; chick survival could only be determined of nests in which at least one egg hatched. Of all pair years, 82% had only one clutch per year, 17% had

Table 1
Definitions, distributions, sample sizes, means, standard deviations (SDs), and ranges of measures of reproductive performance

Reproductive parameter	Definition	Distribution	*N*	Mean ± SD	Range
Laying date	Day first egg was laid (1 April = day 1)	Gaussian	1006	56.4 ± 13.1	26–100
Clutch size	Number of eggs at start of incubation	Poisson	1224	2.68 ± 0.88	1–4
Egg survival	Probability egg survived till hatching	Binomial	881	0.54 ± 0.41	0–1
Chick survival	Probability chick survived till fledging	Binomial	612	0.20 ± 0.32	0–1
Annual fledging production	Annual number of fledglings produced per pair	Poisson	1224	0.23 ± 0.54	0–3

The data set comprises 233 females, 230 males, and 370 pairs measured in 21 different years.

one replacement clutch, and 2% had 2 or more replacement clutches per year.

Statistical procedure

We used generalized linear mixed models (Snijders and Bosker 1999) to account for sources of interdependency between individual measurements. In this data set, there were many repeated measurements of the same male or female (on average 5.3 cases per individual, range 1–19), which might be intercorrelated; similarly, measurement of different individuals in the same year might be intercorrelated due to between-year differences. We modeled female identity, male identity, and year identity as cross-classified random effects with individual measurements nested within these random effects. The advantages of the random effects approach we use in this study over more conventional approaches to the analyses of age-dependent reproduction are discussed in more detail in van de Pol and Verhulst (2006). Analyses were performed with MLwiN 2.0 (Rasbash et al. 2004).

We entered linear and quadratic effects of pair-bond duration as well as male and female breeding experience as continuous variables into the model. Collinearity between pair-bond duration and the breeding experience of both sexes might make correct model selection problematic (Graham 2003); therefore, we estimated the effect of pair-bond duration in the presence of any effects of breeding experience of the male and the female. Nonsignificant quadratic terms of age-related parameters were removed from the model, but linear terms were always retained in the model, significant or not.

For the final model, we tested whether other curvilinear functions of age-related parameters (inverse and logarithmic functions) fitted better than linear and quadratic effects (cf., Sydeman et al. 1991). Both a linear, inverse, and logarithmic functional response can depict a monotonic increase in reproductive performance, but a quadratic functional response might indicate a decline after an initial increase. We compared these nonnested curvilinear models by using Akaike's Information Criteria (AIC) scores (Akaike 1973). Models within 2 AIC units of each other were considered equally well supported (Burnham and Anderson 2002).

Effects of pair-bond duration in cross-sectional analyses can be caused by within-pair changes as well as by progressive changes in the phenotypic composition in the population. Therefore, we verified whether individuals or pairs that disappeared from the population (either due to divorce or due to mortality) were of high or low phenotypic quality. More specifically, by entering the maximum pair-bond duration of a pair as a covariate to the final model, we were able to specifically test for a between-pair covariation with the reproductive parameter of interest (sensu van de Pol and Verhulst 2006; for an alternative approach, see Black 2001). For example, a positive correlation between the maximum pair-bond duration of a pair and its overall fledgling production indicates that high-quality pairs stayed together for longer.

Experimental mate removal: manipulating pair-bond duration

We manipulated pair-bond duration by removing randomly selected partners (either the male [$n = 9$] or the female [$n = 11$]) during the incubation phase; the removed birds were kept in captivity permanently. By removing one of the partners and letting the remaining partners remate with another bird, the pair-bond duration of the newly mated partners was reset to one. This experiment was conducted as part of an earlier study on the mechanisms of territory acquisition by nonbreeders (Bruinzeel and van de Pol 2004) and on the competitive abilities of the removed breeders on neutral grounds in captivity (Bruinzeel et al. 2006). In contrast to these previous studies, here we used the experimental removal of breeders to investigate what happened to the individuals that were not experimentally removed.

When manipulating pair-bond duration in the field, it is not possible to keep all other age-related traits constant. More specifically, the nonremoved individuals increased normally in age and breeding experience during the experiment; and by inducing remating, not only the pair-bond duration but also the age and the breeding experience of the partners were manipulated. However, changes in age and breeding experience of the nonremoved partner during the experiment were the same for all individuals and were rather small compared with the manipulated difference in pair-bond duration and breeding experience between the old and new partner. Furthermore, by removing both males and females, we were able to disentangle effects of manipulated changes in pair-bond duration, age, or breeding experience. For example, when only effects of male age or breeding experience would affect reproductive performance, then we would expect that only the experimental removal of a male partner and subsequent remating would affect the reproductive performance of the nonremoved partner (the removal of a female partner does not manipulate male breeding experience). Alternatively, when pair-bond duration affects reproductive performance, we would expect that the removal of males as well as females would result in a change in reproductive performance of the new partner, as pair-bond duration is manipulated in both types of removals.

We followed the change in reproductive performance of the nonremoved individual in the 4 years before and the 4 years after the removal and related this to the change in pair-bond duration. Measurements of reproductive performance were corrected for variation between years and territory quality by subtracting the average value per year and territory type from each measurement. Experiments were performed in 1998 and 1999 under license number 2099 of the Animal Experiment Board of the University of Groningen; detailed methods are described in Bruinzeel and van de Pol (2004). The individuals of whom the partners were removed were excluded from the larger data set described in the previous section. Regrettably, in the years after the experiment, the salt marsh flooded during the incubation phase, which severely reduced sample sizes for most reproductive parameters with exception of laying date. Therefore, only this reproductive parameter could be analyzed.

RESULTS

Observational patterns

The aim of combined analyses of natural and experimental variation in pair-bond duration and reproductive performance is to compare both patterns. However, experiments were carried out on individuals that did not change territory; therefore, we first analyzed the association between pair-bond duration and territory quality in the observational data set. Pair-bond duration did not affect the probability of owning a high-quality territory (Table 2A). However, inexperienced males and females had a much lower probability of owning a high-quality territory than experienced males and females (Figure 1 and Table 2A). A quadratic functional response of male breeding experience resulted in higher model support (ΔAIC = 2.3) than an inverse or logarithmic function of male breeding experience. This suggests that the probability of owning a high-quality territory

Table 2

Final models of analyses of effects of age-related parameters on reproductive performance: (A) probability of owning a high-quality territory, (B) laying date, (C) clutch size, (D) egg survival, (E) chick survival, and (F) annual fledgling production

Fixed effects	β	SE (β)	ΔDeviance	df	*P*
(A) Dependent variable: probability owning high-quality territory (logit link function)					
Final model			1612	1216	
Intercept	−1.39	0.21		1	
♀ Breeding experience	0.166	0.049	11.27	1	0.001
♀ Breeding experience^2	−0.0054	0.0027	4.13	1	0.042
♂ Breeding experience	0.156	0.053	8.76	1	0.003
♂ Breeding experience^2	−0.0097	0.0030	10.44	1	0.001
Pair-bond duration	0.163	0.270	0.15	1	0.69
Random effects: $\sigma^2_{year} = 0.051 \pm 0.038$, $\sigma^2_{residual} = 1$[a]					
(B) Dependent variable: laying date (identity link function)					
Final model			7514	996	
Intercept	61.2	1.4		1	
Territory quality	−2.89	1.13	6.42	1	0.011
♀ Breeding experience	−0.082	0.140	0.34	1	0.56
♂ Breeding experience	−0.404	0.134	9.09	1	0.003
Pair-bond duration	−0.823	0.299	6.23	1	0.013
Pair-bond duration^2	0.067	0.022	7.44	1	0.006
Random effects: $\sigma^2_{year} = 7.3 \pm 3.7$, $\sigma^2_{females} = 51.3 \pm 9.0$, $\sigma^2_{males} = 8.7 \pm 6.4$, $\sigma^2_{residual} = 102.8 \pm 5.6$					
(C) Dependent variable: clutch size (log link function)					
Final model			395	1214	
Intercept	0.902	0.052		1	
Territory quality	0.061	0.027	4.74	1	0.029
♀ Breeding experience	0.0011	0.0049	0.05	1	0.82
♂ Breeding experience	0.023	0.011	4.02	1	0.044
♂ Breeding experience^2	−0.0011	0.0005	4.99	1	0.025
Pair-bond duration	0.0043	0.0080	0.28	1	0.60
Random effects: $\sigma^2_{year} = 0.0024 \pm 0.0020$, $\sigma^2_{females} = 0.0015 \pm 0.0016$, $\sigma^2_{males} = 0.0013 \pm 0.0009$, $\sigma^2_{residual} = 1$[a]					
(D) Dependent variable: egg survival (logit link function)					
Final model			2652	871	
Intercept	0.30	0.33		1	
Territory quality	0.32	0.13	5.77	1	0.016
♀ Breeding experience	−0.0004	0.018	0.001	1	0.97
♂ Breeding experience	−0.0014	0.018	0.007	1	0.93
Pair-bond duration	0.183	0.062	9.42	1	0.002
Pair-bond duration^2	−0.013	0.005	7.61	1	0.006
Random effects: $\sigma^2_{year} = 1.59 \pm 0.61$, $\sigma^2_{females} = 0.14 \pm 0.10$, $\sigma^2_{males} = 0.19 \pm 0.10$, $\sigma^2_{residual} = 1$[a]					
(E) Dependent variable: chick survival (logit link function)					
Final model			1189	607	
Intercept	−1.81	0.32		1	
Territory quality	0.90	0.19	23.4	1	<0.001
♀ Breeding experience	−0.014	0.028	0.25	1	0.62
♂ Breeding experience	−0.059	0.034	3.11	1	0.078
Pair-bond duration	−0.016	0.042	0.15	1	0.70
Random effects: $\sigma^2_{year} = 0.83 \pm 0.45$, $\sigma^2_{females} = 0.14 \pm 0.15$, $\sigma^2_{males} = 0.14 \pm 0.14$, $\sigma^2_{residual} = 1$[a]					
(F) Dependent variable: annual fledgling production (log link function)					
Final model			678	1214	
Intercept	−2.87	0.43		1	
Territory quality	0.94	0.16	34.5	1	<0.001
♀ Breeding experience	0.004	0.025	0.02	1	0.89
♂ Breeding experience	−0.013	0.027	0.23	1	0.63
Pair-bond duration	0.233	0.106	4.77	1	0.029
Pair-bond duration^2	−0.022	0.010	4.48	1	0.034
Random effects: $\sigma^2_{year} = 1.63 \pm 0.82$, $\sigma^2_{females} = 0.22 \pm 0.12$, $\sigma^2_{males} = 0.05 \pm 0.06$, $\sigma^2_{residual} = 1$[a]					

SE, standard error; df, degrees of freedom.

[a] Note that in models that use a log or logit link function, the residual variance is constrained to be 1.

decreased again for males with more than 10 years of breeding experience, whereas this decline was not apparent in very experienced females. Because we were primarily interested in the occurrence of improvement independent of the environment in which individuals lived, we statistically corrected for territory quality in all other analyses.

Both pair-bond duration and male breeding experience were associated with several reproductive parameters; female breeding experience did not affect any of the reproductive parameters. Lay date was associated with both pair-bond duration and male breeding experience (Table 2B). Pairs with long pair-bond duration laid earlier than newly formed pairs;

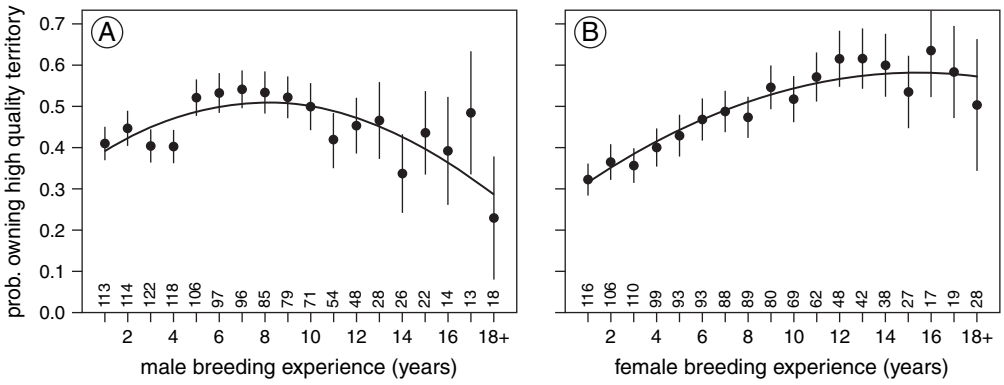

Figure 1
Effects of (A) male breeding experience and (B) female breeding experience on the probability of owning a high-quality territory, adjusted for all other fixed and random effects in the final models (Table 2A). On average, 45% of the population owns a high-quality territory. Note that very experienced individuals (18 years and more) were grouped for graphical reasons—analyses were performed on ungrouped values. Sample sizes are above the *x* axis.

however, pairs that had been together for over 10 years laid very late (Figure 2B). Laying dates were 0.4 days earlier for each year of breeding experience of the male (Figure 2A). Clutch size was associated with male breeding experience only (Table 2C). Clutch size was slightly higher for experienced males than for first time breeders and seemed to be lower again for very experienced males (Figure 2C); however, overall differences were small (0.3 eggs). Egg survival was low for newly formed pairs, highest for pairs that had been together for 5–7 years, but declined again for pairs that had been together for very long (Figure 2D and Table 2D). Chick survival was not significantly associated with any of the age-related parameters, although there was a suggestion that chick survival declined with male breeding experience ($P = 0.078$, Table 2E). Annual reproductive output was associated only with pair-bond duration. Newly formed pairs produced few fledglings each year, production increased strongly after their first year together and remained high up to the seventh year of the pair-bond; however, very old pairs performed extremely poor (Figure 2F and Table 2F). Annual fledgling production was not associated with male breeding experience (Figure 2E and Table 2F), probably because the strong positive effects on lay date were canceled out by the negative trend with chick survival. Interactive effects of male and female breeding experience (the sum or product of male and female breeding experience) did also not affect final reproductive output (both $P > 0.4$).

Quadratic relationships always explained the observed relationships between age-related parameters and reproductive parameters at least as good as inverse or logarithmic relationships. The quadratic effect of pair-bond duration on lay date, egg survival, and annual fledgling production described the data better than other curvilinear relationships (all $\Delta AIC > 2$). This suggests that lay date, egg survival, and fledgling production first increased with pair-bond duration, peaked at intermediate pair-bond duration, and subsequently declined (and did not level off) at very long pair-bond duration. Pairs that broke up early (low maximum pair-bond duration) were of similar quality as pairs that stayed together for very long (high maximum pair-bond duration). This result follows from the observation that maximum pair-bond duration did not covary with lay date ($\beta = 0.32 \pm 0.22$; $\chi^2 = 2.4$, $P = 0.12$), egg survival ($\beta = 0.01 \pm 0.03$; $\chi^2 = 0.03$, $P = 0.86$), or annual fledgling production ($\beta = 0.02 \pm 0.03$; $\chi^2 = 0.48$, $P = 0.49$) when evaluated in the final models presented in Table 2. As there was no evidence in favor of selective disappearance of high- or low-quality pairs, older pairs must have performed better because they improved during their pair-bond.

Experimental mate removal

We compared the changes in lay date in the 4 years before and the 4 years after the mate removal experiment. We calculated the individual change (slopes) in laying dates for the 4 years before and after the divorce separately (Figure 3A). Slopes were similar between the sexes before and after divorce (both $P > 0.2$). Slopes before the divorce were not significantly different from zero (average = 1.4 ± 1.2 days/year, $t_{1,12} = 1.16$, $P = 0.27$). However, slopes after divorce differed significantly from zero (average = -4.6 ± 1.6 days/year, $t_{1,12} = -2.94$, $P = 0.012$) and differed significantly from the same individuals' slopes before the divorce (Δslopes = -6.0 ± 2.0 days/year, paired $t_{1,12} = -3.06$, $P = 0.010$). This indicates that the same nonremoved individuals, which had a constant laying date in the 4 years before the divorce, started advancing their laying dates after they remated with a new partner, independent of the sex of the nonremoved bird. The age and breeding experience of the new partners were not systematically higher or lower than the age of the removed old partners (age: paired $t_{1,12} = -0.05$, $P = 0.96$; breeding experience: paired $t_{1,12} = -0.58$, $P = 0.57$). Therefore, the change in slopes before and after the divorce, which was similar in both sexes, cannot easily be explained by a change in age or breeding experience of either males or females. Laying dates were expressed relative to the mean laying date for that year and territory quality, and consequently, the experimental effect can also not be explained by general environmental differences between years or territories.

The relationship between pair-bond duration and laying date in the observational data set suggested that experimental mate removal should also result in an immediate cost of divorce as it results in a decrease in pair-bond duration (except for newly formed pairs). Therefore, we also calculated the absolute change in standardized laying dates from the year before to the year after the divorce. Based on the observational patterns (Figure 2B), we expected that new pairs would not benefit from divorce (no change in lay date), pairs that had been together for up to 10 years should suffer a cost of divorce (delayed egg laying), but that very old pairs should

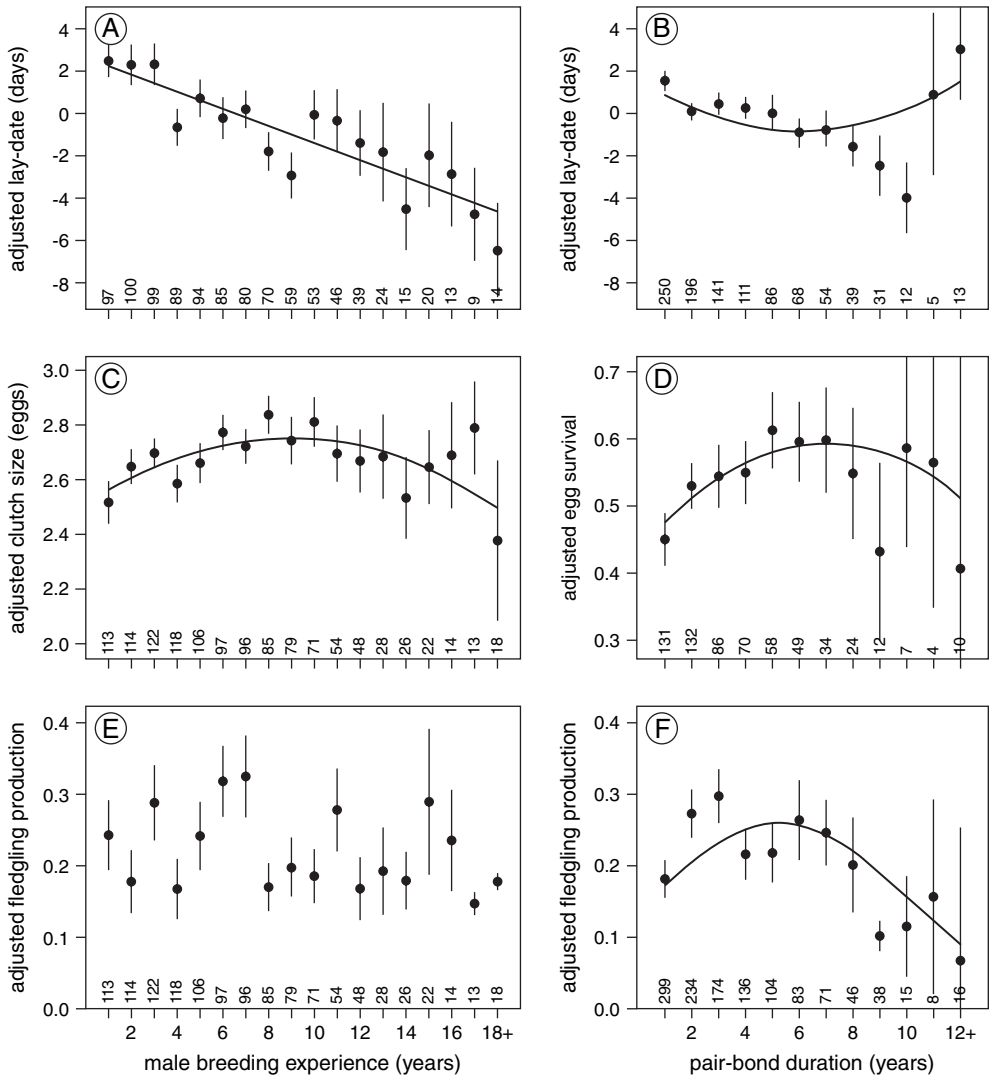

Figure 2
Effect of male breeding experience on (A) laying date, (C) clutch size, and (E) annual fledgling production and the effect of pair-bond duration on (B) laying date, (D) egg survival, and (F) annual fledgling production. Reproductive parameters were adjusted for all other fixed and random effects in the final models (Table 2B–F). Note that very experienced individuals and pairs were grouped for graphical reasons—analyses were performed on ungrouped values. Sample sizes are above the *x* axis.

even benefit from divorce (advance egg laying). The mate removal experiment was qualitatively exact in agreement with expectation, although the quantitative changes were much stronger than expected (up to 1 month delay or advancement, Figure 3B). The change in laying date was quadratically proportional to the change in pair-bond duration (Table 3). Note that the change in pair-bond duration is equivalent to the pair-bond duration before the experiment minus one, as all individuals remated with new partners. The effect of pair-bond duration before the experiment on changes in laying date differed between the sexes, with male removals more often resulting in delayed laying when young pairs were manipulated. The pair-bond duration before the divorce explained 60% of the variance in both sexes in changes in laying date due to the divorce. Changes in other age-related traits due to the divorce, especially male breeding experience, did not correlate with the change in laying date (Table 3). The observed patterns were probably not the result of problems associated with finding a new mate in time for the next breeding season, as most individuals remated within a week (Bruinzeel and van de Pol 2004). Furthermore, a comparison between changes in lay date between the year before the divorce and 2 years after the divorce resulted in very similar patterns as in Figure 3B (results not shown).

DISCUSSION

Our finding that reproductive performance improves during the pair-bond is in agreement with the observation that remated pairs outperformed newly formed pairs in another population of oystercatchers (Harris et al. 1987), as well as in many other bird species (Fowler 1995; Ens et al. 1996). Reproductive output improved strongly during the first years of the pair-bond; however, pairs that had been together for very long performed very badly (Figure 2F). These patterns remained after controlling for effects of breeding experience of the male and female. The association between pair-bond duration and reproductive performance in the cross-sectional analyses was not caused by high- or low-quality pairs breaking up earlier but due to within-pair changes. The initial increase in annual fledgling production during the pair-bond seemed to have resulted from an advance of timing of egg laying and an increase in egg survival during the pair-bond (Figure 2B,D). About a dozen other studies have also reported a negative association between laying date and pair-bond duration (Fowler 1995). These same studies did not investigate or report a positive association between egg survival and pair-bond duration; however, such an association was nonetheless likely to be present in these studies, as eggs laid early in the season

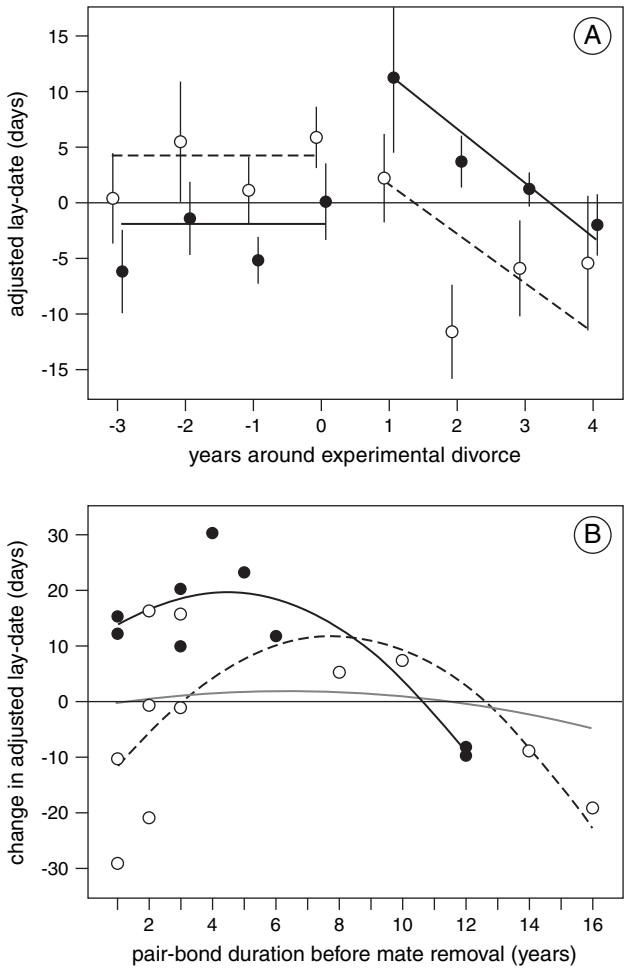

Figure 3
Changes in laying date of the remated individuals in (A) the 4 years preceding and in the 4 years after the experimental mate removal and (B) from the year before to the year after the experimental mate removal. In (A), closed dots and continuous lines give values and average slopes of the individual changes in laying dates (before and after removal) for remated females (males removed, $N = 6$); open dots and dashed lines are used for males (females removed, $N = 7$). The experimental removal and subsequent remating with a different partner occurred in year 0, after egg laying. In (B), closed dots and continuous line give values and model fit for remated females (males removed, $N = 9$); open dots and dashed lines are used for remated males (females removed, $N = 11$). The grey line represents the expected relationship based on the observational data (see Figure 2B). All laying dates are adjusted for year and territory quality effects, and we subtracted each individual's overall average laying date. Sample sizes in (B) were higher due to missing values in (A).

usually have a higher chance of survival in most birds (Perrins 1970).

Several other studies have conducted mate removal experiments (e.g., Sherry and Holmes 1989; Otter and Ratcliffe 1996); however, to our knowledge, this is the first study that manipulated pair-bond duration and analyzed it in that context. The manipulated change in pair-bond duration due to the experimental mate removal and subsequent remating strongly affected the reproductive performance of the nonremoved partner (Figure 3). Because it is impossible to manipulate pair-bond duration and keep the age and breeding experience of the new partners constant in a field situation, it was crucial to remove both males and females. For example, if only male breeding experience affected reproductive performance, we would have expected no effect on the nonremoved individual in cases where a female partner was removed, as the removal of a female partner does not manipulate the male breeding experience. However, the removal of either a male or a female partner affected the reproductive performance of the nonremoved partner in a qualitatively similar way, although the strength of response differed somewhat between the sexes (Figure 3B). Therefore, the combined observational patterns and experimental manipulation of pair-bonds provide strong support for a causal relationship between pair-bond duration and reproductive performance.

A causal effect of pair-bond duration on reproductive performance implies that a divorce incurs an immediate cost, as a result of a loss in mate familiarity (except for newly formed pairs). The mate removal experiment confirmed that the change in laying date was dependent on the reduction in pair-bond duration due to the divorce. Although the result of the experiment qualitatively supported the observational data, the quantitative changes in laying date were much stronger in the experiment. We do not understand why changes after experimental remating were much stronger than natural patterns. Potentially the act of removal itself may have resulted in a trauma to the nonremoved partner. We did not directly control for such a "trauma effect," as we had no control group in which the same individuals were first removed and later released. However, we think it is unlikely that a trauma effect would have resulted in a higher cost of divorce, as most birds remated within a week (Bruinzeel and van de Pol 2004), and it seems unlikely that this would have affected their laying date almost a year later. Moreover, it is not obvious why a hypothetical trauma effect would vary quadratically with pair-bond duration.

Interestingly, both the observational and experimental data suggest it would be beneficial for very old pairs to divorce. However, the probability of divorce was independent of pair-bond duration or any other age-related trait (logistic regression, all $P > 0.10$, $N = 1224$ pair years). Possibly, old pairs do not divorce more often because the number of vacancies is limited in a natural situation; consequently, initiating divorce could result in becoming a nonbreeder. However, this does not explain why in another population of oystercatchers it was found that newly formed pairs divorced most often (Harris et al. 1987).

After the experimentally induced remating, the newly formed pairs advanced their timing of reproduction with 2 weeks over the next 4 years. Long-term gradual improvements of reproductive performance are consistent with a mate familiarity effect (Black 1996b) and were also observed after natural divorce in oystercatchers (Heg et al. 2003) and short-tailed shearwaters (*Puffinus tenuirostris)* (Bradley et al. 1990). Studies reporting improvements of reproductive performance associated with age-related traits usually report a strong initial improvement early in life or during the pair-bond. This observation is consistent with the idea that individuals or pairs have to become familiar with specific reproductive tasks (e.g., mate coordination) and resembles classical learning curves (Forslund and Pärt 1995). Within-pair improvements in laying date were not limited to the first few years of the pair-bond, but pairs gradually advanced laying up to their ninth year of the pair-bond. Most strikingly, after an initial improvement, pairs deteriorated strongly in reproductive performance during the remainder of their pair-bond. Pairs that had been together for over 12 years produced much less offspring each year than young pairs (Figure 2F). A decline in reproductive performance at high values of pair-bond duration was not a statistical artifact because also in the experiment a quadratic relationship was observed. Furthermore, a decline at long pair-bond duration does not seem to be an exceptional pattern in studies that report effects of pair-bond duration on reproductive

Table 3

Effects of experimentally induced changes in age-related parameters on the change in laying date with the old partner in the year of divorce (mate removal) and new partner in the year after divorce

Parameter	β	SE (β)	df	*F*	*P*
Final model ($N = 20$, $R^2 = 0.60$)			4,15	8.0	0.001
Intercept	−18.9	6.4	1	0.6	0.44
Sex	28.8	7.3	1	15.7	0.001
Pair-bond duration	7.9	2.4	1	7.9	0.013
Pair-bond duration^2	−0.51	0.15	1	12.2	0.003
Sex × pair-bond duration	−3.4	1.1	1	9.5	0.008
Rejected from final model					
Δ Age old and new partner	0.5	0.6	1	0.1	0.75
Sex × Δ age old and new partner	−0.5	1.2	1	0.2	0.65
Δ Breeding experience old and new partner	0.5	0.6	1	0.3	0.58
Sex × Δ breeding experience old and new partner	−0.4	1.1	1	0.1	0.73
Sex × pair-bond duration^2	−0.04	0.34	1	0.01	0.91
Territory quality	−4.3	5.2	1	0.7	0.42

SE, standard error; df, degrees of freedom. Sex refers to the sex of the removed partner; territory quality refers to the territory quality in which the experiment was performed. Pair-bond duration is the pair-bond duration before the experiment, which is equivalent to the change in pair-bond duration as all remaining individuals started out with a new partner.

performance (Ens et al. 1996). Possibly, the decline in reproductive performance is an inescapable cost of the high reproductive performance in the first years of the pair-bond duration and can be interpreted as reproductive senescence.

The behavioral changes that underlie the change in reproductive performance during the pair-bond are probably not primarily determined by changes in the individual behavior of the male or the female. More likely, they are the result of synergistic effects between male and female (i.e., how they function as a pair). Possible candidate behaviors must therefore be the result of the actions of both the male and the female, in particular the responses to each other (i.e., improved intrapair coordination or cooperation). The analyses of different measures of reproductive performance offers further insight in the underlying mechanisms of overall improvement of reproductive output. The strong effect of pair-bond duration on annual fledgling production can largely be explained by the combined effects of pair-bond duration on laying date and egg survival. This suggests that the behavioral mechanisms underlying these improvements are acting during the prelaying and egg incubation period. Prelaying candidate behaviors could include the mate coordination and cooperation in defending a rich feeding territory, as laying date in oystercatchers is affected by access to food (Heg 1999). Egg survival is mainly determined by egg predation (predominantly by gulls and possibly rats). To protect the eggs, both the male and the female have to sit on the nest for almost 12 h a day each, consequently, oystercatchers are time stressed as they can only use half the usual available time during low tide to feed. Possible candidate behaviors during egg incubation could involve the coordination of incubation bouts and the efficiency of the division of labor between both sexes. Increasing mate familiarity during a pair-bond could help in fine tuning such cooperative behavior.

Other studies that have investigated behaviors that could be responsible for a mate familiarity effect offer only a few hints. Various behaviors in Pinyon jays (*Gymnorhinus exanocephalus*) and dominance positions in Bewick's swans (*Cygnus columbianus*) were not associated with pair-bond duration (Marzluff et al. 1996; Reese et al. 1996). However, a study on Barnacle geese (*Branta leucopsis*) suggested that long-term pairs occupy better positions in feeding flocks, possibly as a result of increased dominance during the pair-bond (Black et al. 1996). A study on Zebra finches (*Taeniopygia guttata*) suggested that incubation behavior might improve during the pair-bond (Delesalle 1986). However, showing that certain behaviors are associated with pair-bond duration does not prove causation for the same reasons that an association between reproduction and pair-bond duration does not prove a mate familiarity effect (see introduction). It is clear that there is still a long way to go from current phenomenological descriptions of improved reproductive success during the pair-bond to a mechanistic understanding of how pairs achieve such an increase in reproductive performance. As such, we fully support Ens et al. (1996) plea for more detailed behavioral studies on this topic.

So far we assumed, as did most studies, that all individual or pair improvements are due to an accumulation of different types of experience (Curio 1983). An alternative explanation would be that individuals restrain themselves at a younger age for optimality reasons when current reproduction is traded off against future reproduction and survival declines with age (Pianka and Parker 1975; Curio 1983). However, we consider this explanation unlikely because there is no evidence for age-dependent mortality among adult oystercatchers (Schnakenwinkel 1970). More importantly, the restraint hypothesis only predicts that individuals should optimize their current reproductive effort in relation to their life expectancy, and therefore, the restraint hypothesis cannot explain why reproductive effort should be optimized in relation to pair-bond duration. Nonetheless, reasons for adjusting reproductive effort in relation to pair-bond duration might exist. Roberts and Sherratt (1998) suggested cooperation can evolve in individuals that gradually increase their investments in repeated reciprocal interactions ("raise-the-stakes" strategies), as this strategy is robust against individuals that try to exploit their partners. Several experiments have shown that humans used such strategies to improve cooperation between unrelated partners (Wedekind and Milinski 1996; Roberts and Renwick 2003). Consequently, the idea that pairs might have restrained themselves from producing more offspring at the beginning of the pair-bond (as a result of a conflict in a cooperation game) might be an alternative explanation for a mate familiarity effect.

Studies on divorce and the evolution of monogamy have largely focused on 2 types of explanations for why long-term

mate fidelity exists (Black 1996a): 1) potential costs of divorce (finding a new partner, losing a territory) and 2) potential benefits of maintaining pair-bonds (due to mate familiarity effects). A comparison between species seems to confirm the important role of mate familiarity effects on divorce decisions: studies that reported effects of pair-bond duration on reproductive success were mainly on bird species with low divorce rates (5.8 ± 2.1% annually, $N = 8$), whereas studies that failed to find an effect of pair-bond duration were on bird species with high divorce rates (18.7 ± 6.7%, $N = 7$, calculated from data in Ens et al. 1996). However, both types of explanations for why long-term mate fidelity exists are not mutually exclusive. For example, in oystercatchers, the availability of breeding territories is strongly limited; initiating a divorce by deserting your mate is thought to be very risky and can result in losing your territory, especially for breeders in high-quality habitat (Heg et al. 2003).

Finally, the important role of the pair-bond suggests that reproductive performance is not purely an individual trait, but that reproductive performance is the result of an interaction between the phenotypes of the male and the female. Synergistic effects during the pair-bond were very strong in this study, and such strong effects do not seem to be uncommon in monogamous bird species (Ens et al. 1996). This poses the question to what extent synergistic effects might interact with natural selection shaping individual life history patterns (Moore et al. 1998). Much more work is needed to address these types of questions, but given the complex nature of such question, experimental approaches such as this study seem to be crucial.

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REFERENCES

Akaike H. 1973. Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Csaki N, editors. Proceedings of the 2nd International Symposium on Information Theory. Budapest, Hungary: Akademiai Kiado. p 267–81.

Axelrod RM. 1984. The evolution of cooperation. New York: Basic Books

Black JM. 1996a. Partnerships in birds. The study of monogamy. Oxford: Oxford University Press.

Black JM. 1996b. Introduction: pair bonds and partnerships. In: Black JM, editor. Partnerships in birds. The study of monogamy. Oxford: Oxford University Press. p 3–20.

Black JM. 2001. Fitness consequences of long-term pair bonds in barnacle geese: monogamy in the extreme. Behav Ecol 12:640–5.

Black JM, Choudhury S, Owen M. 1996. Do Barnacle geese benefit from lifelong monogamy? In: Black JM, editor. Partnerships in birds, the study of monogamy. Oxford: Oxford University Press. p 91–117.

Bradley JS, Wooller RD, Skira IJ, Serventy DL. 1990. The influence of mate retention and divorce upon reproductive success in short-tailed shearwaters *Puffinus tenuirostris*. J Anim Ecol 59:487–96.

Bruinzeel LW. 2004. Search, settle, reside & resign [PhD dissertation]. Groningen: University of Groningen. p 1–123.

Bruinzeel LW, van de Pol M. 2004. Site attachment of floaters predicts success in territory acquisition. Behav Ecol 50:290–6.

Bruinzeel LW, van de Pol M, Trierweiler C. 2006. Competitive abilities of oystercatchers (*Haematopus ostralegus*) occupying territories of different quality. J Ornithol 147:457–63.

Burnham KP, Anderson DR. 2002. Model selection and multimodel inference. New York: Springer Verlag.

Cézilly F, Nager RG. 1995. Age and breeding performance in monogamous birds: the influence of pair stability. Trends Ecol Evol 11:27.

Clutton-Brock TH. 1988. Reproductive success. Studies of individual variation in contrasting breeding systems. Chicago: University of Chicago Press.

Curio E. 1983. Why do young birds reproduce less well? Ibis 125:400–4.

Delesalle VA. 1986. Division of parental care and reproductive success in the zebrafinch, *Taeniopygia guttata*. Behav Process 12:1–22.

Ens BJ, Choudhury S, Black JM. 1996. Mate fidelity and divorce in monogamous birds. In: Black JM, editor. Partnerships in birds, the study of monogamy. Oxford: Oxford University Press. p 344–401.

Ens BJ, Kersten M, Brenninkmeijer A, Hulscher JB. 1992. Territory quality, parental effort and reproductive success of oystercatchers (*Haematopus ostralegus*). J Anim Ecol 61:703–15.

Forslund P, Pärt T. 1995. Age and reproduction in birds—hypotheses and tests. Trends Ecol Evol 10:374–8.

Fowler GS. 1995. Stages of age-related reproductive success in bird: Simultaneous effects of age, pair bond duration and reproductive experience. Am Zool 35:318–28.

Graham MH. 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84:2809–15.

Harris MP, Safriel UN, Brooke M de L, Britton CK. 1987. The pair bond and divorce among oystercatchers *Haematopus ostralegus* on Skokholm Island, Wales. Ibis 129:45–57.

Heg D. 1999. Life-history decisions in oystercatchers [PhD dissertation]. Groningen, The Netherlands: University of Groningen. p 1–254.

Heg D, Bruinzeel LW, Ens BJ. 2003. Fitness consequences of divorce in the oystercatcher, *Haematopus ostralegus*. Anim Behav 66:175–84.

Heg D, Dingemanse NJ, Lessells CM, Mateman AC. 2000. Parental correlates of offspring sex ratio in Eurasian oystercatchers. Auk 117:980–6.

Heg D, Ens BJ, Burke T, Jenkins L, Kruijt JP. 1993. Why does the typically monogamous Oystercatcher (*Haematopus ostralegus*) engage in extra-pair copulations? Behaviour 126:247–89.

Heg D, Ens BJ, van der Jeugd HP, Bruinzeel LW. 2000. Local dominance and territorial settlement of nonbreeding oystercatchers. Behaviour 137:473–530.

Marzluff JM, Woolfenden GE, Fitzpatrick JW, Balda RP. 1996. Breeding partnerships in two new world jays. In: Black JM, editor. Partnerships in birds. The study of monogamy. Oxford: Oxford University Press. p 138–61.

Moore AJ, Brodie ED, Wolf JB. 1998. Interacting phenotypes and the evolutionary process: direct and indirect genetic effects of social interactions. Evolution 51:1352–62.

Newton I. 1989. Lifetime reproduction in birds. London: Academic Press.

Otter K, Ratcliffe L. 1996. Female initiated divorce in a monogamous songbird: abandoning mates for males of higher quality. Proc R Soc Lond B Biol Sci 263:351–4.

Pärt T, Forslund P. 1995. Age and breeding performance in monogamous birds: the influence of mate experience. Trends Ecol Evol 11:220.

Perrins CM. 1970. The timing of birds' breeding season. Ibis 112: 224–55.

Pianka ER, Parker WS. 1975. Age-specific reproductive tactics. Am Nat 109:453–64.

Prater T, Marchant J, Vuorinen J. 1987. Guide to the identification and ageing of holarctic waders. Tring, Hertfordshire: British Trust for Ornithology.

Pyle P, Sydeman WJ, Hester M. 2001. Effects of age, breeding experience, mate fidelity and site fidelity on breeding performance in a declining population of Cassin's auklets. J Anim Ecol 70:1088–97.

Rasbash J, Steele F, Browne W, Prosser B. 2004. A user's guide to MLwiN—version 2.0. London: Centre for Multilevel Modelling.

Reese EC, Lievesley P, Pettifor RA, Perrins C. 1996. Mate fidelity in swans: an interspecific comparison. In: Black JM, editor. Partnerships in birds, the study of monogamy. Oxford: Oxford University Press. p 118–37.

Roberts G, Renwick JS. 2003. The development of cooperative relationships: an experiment. Proc R Soc Lond B Biol Sci 270:2279–83.
Roberts G, Sherratt TN. 1998. Development of cooperative relationships through increasing investment. Nature 394:175–9.
Schnakenwinkel G. 1970. Studien an der population des Austernfischers auf Mellum. Vogelwarte 25:337–55.
Sherry WT, Holmes RT. 1989. Age-specific social dominance affects habitat use by breeding American redstarts (*Setophaga ruticilla*): a removal experiment. Behav Ecol Sociobiol 25:327–33.
Snijders TAB, Bosker RJ. 1999. Multilevel analysis: an introduction to basic and advanced multilevel modeling. London: SAGE Publications.
Sydeman WJ, Huber HR, Emslie SD, Ribic CA, Nur N. 1991. Age-specific weaning success of northern elephant seals in relation to previous breeding experience. Ecology 72:2204–17.
van de Pol M, Bakker T, Saaltink DJ, Verhulst S. 2006. Rearing conditions determine offspring survival, independent of egg quality: a cross-foster experiment with oystercatchers. Ibis 148:203–10.
van de Pol M, Bruinzeel LW, Heg D, van der Jeugd HP, Verhulst S. 2006. A silver spoon for a golden future: long-term effects of natal origin on fitness prospects of Oystercatchers. J Anim Ecol 75:616–26.
van de Pol M, Verhulst S. 2006. Age-dependent traits: a new statistical method to separate within and between-individual effects. Am Nat 167:764–71.
Wedekind C, Milinksi M. 1996. Human cooperation in the simultaneous and the alternating Prisoner's Dilemma: Pavlov versus Generous Tit-for-Tat. Proc Natl Acad Sci USA 93:2686–9.
Zwarts L, Hulscher JB, Koopman K, Zegers P. 1996. Discriminating the sex of oystercatchers *Haematopus ostralegus*. Ardea 84A:1–12.